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FOR THE RECORD

Crystallization and preliminary structural analysis of catalase A from *Saccharomyces cerevisiae*

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Abstract: Yeast peroxisomal catalase A, obtained at high yields by over expression of the C-terminally modified gene from a 2μ-plasmid, has been crystallized in a form suitable for high resolution X-ray diffraction studies. Brownish crystals with bipyrimidal morphology and reaching ca. 0.8 mm in size were produced by the hanging drop method using ammonium sulphate as precipitant. These crystals diffract better than 2.0 Å resolution and belong to the hexagonal space group $\mathbf{P6_122}$ with unit cell parameters **a** = **b** = 184.3 Å and **c** = 305.5 Å. An X-ray data set with 76% completeness at 3.2 Å resolution was collected in a rotating anode generator using mirrors to improve the collimation of the beam. An initial solution was obtained by molecular replacement only when using a beef liver catalase tetramer model in which fragments with no sequence homology had been omitted, about 150 residues per subunit. In the structure found a single molecule of catalase A (a tetramer with accurate 222 molecular symmetry) is located in the asymmetric unit of the crystal with an estimated solvent content of about 61%. The preliminary analysis of the structure confirms the absence of a carboxy terminal domain as the one found in the catalase from *Penicillium vitalae*, the only other fungal catalase structure available. The NADPH binding site appears to be involved in crystal contacts, suggesting that heterogeneity in the occupancy of the nucleotide can be a major difficulty during crystallization.

Keywords: protein engineering; X-ray analysis; yeast catalase

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Abbreviations: BLC, bovine liver catalase; PMSF, phenyl methyl sulphonyl fluoride; PVC, *Penicillium vitalae* catalase; Sc-ura, 0.67% (w/v) yeast nitrogen base w/o amino acids and uracil, 2% (w/v) glucose, 1% (w/v) mixture of amino acids; YPD, 1% yeast extract, 2% (w/v) meat peptone, 3% (w/v) glucose; YPE, as above but with 3% (v/v) ethanol instead of glucose.

The efficient utilization of hydrogen peroxide both as an electron acceptor and as an electron donor is a unique property of catalases (EC 1.11.1.6) among all other members of the hydroperoxidases family. The yeast *Saccharomyces cerevisiae* contains a peroxisomal and a cytoplasmatic catalase named, respectively, catalases A and T. The two enzymes show about 45% sequence homology and present very similar catalytic properties. The gene coding for the peroxisomal enzyme, *CTA1*, had been cloned (Cohen et al., 1988), over-expressed and modified in an attempt to combine site directed mutagenesis and structural studies (Zamocky et al., 1995). A number of features of catalase A suggested that the structure of this enzyme should provide complementary information with respect to the known catalase structures (Fita et al., 1986; Melik-Adamyan et al., 1986; Murshudov et al., 1992; Bravo et al., 1995; Gouet et al., 1995). The subunit size of catalase A, 515 aminoacids, the fact that it can bind NADPH (Hillar et al., 1994) and a sequence similarity of about 50% with the bovine liver catalase (BLC) indicated that, most probably, it lacks a carboxy terminal domain (Cohen et al., 1988) as that found in catalase from *Penicillium vitalae* (PVC), the only fungal catalase structure presently known but for which the chemical sequence is not yet available (Melik-Adamyan et al., 1986). No indications of modifications or degradations of the heme group as found in the two eukaryotic catalase structures determined, PVC and BLC, have ever been reported for catalase A, and only ferric protoheme IX has been detected by reverse-phase HPLC (Koller et al., unpubl. obs.). The absence of heme mixtures should allow a clearer view of the catalase active center organization. Differences between catalase A and their mammalian relatives extend also to the biochemical and molecular properties. Thus, the relative peroxidatic rates (both with two-electron, as well as with one-electron donors) are distinctly larger for the enzymes of higher eukaryotes, and catalase A appears close to a pure catalase. Isolated dimers of mammalian catalases with limited stability have been described (Aebi et al., 1974), whereas catalase A reflects a single transition upon tetramer dissociation, immediately leading to a complete loss of activity (Zamocky et al., 1995). Finally, catalase A was described as "atypical" due to its very low absorption in the Soret-band region when compared with

the absorption spectra of most catalases (Seah et al., 1973). The high-resolution X-ray crystal determination of catalase A, initiated in this work, should enlarge our understanding of catalases, also providing the structural basis for the peculiarities of this yeast enzyme.

Results and discussion: *Expression and purification of protein CatA modC*: The yeast strain GC1-8B (leu2 trp1 ura3 ctt-1 cta1-2), transformed with the shuttle vector YEP 352-E (Binder et al., 1971), which contains the C-terminally modified CTA1 gene, with the two C-terminal residues of the gene product -KF exchanged to -RHHHF (referred to as CatA modC), under the control of its own promotor (Zamocky et al., 1995) was grown over night in *Sc-ura*, then in YPD until the glucose concentration was less than 0.1%, then for a further 48 h at YPE. Cells were harvested at 6,000 × *g* for 25 min.

The cells were resuspended in 50 mM sodium phosphate, pH 8.0, 0.5 mMEDTA, 70 μg/mL PMSF, 1 μg/mL each of leupeptin and pepstatin A, then disrupted with glass beads in a Braun cell homogenizer (5 × 45 s). The supernatant was consecutively precipitated, first by addition of 1/5 volume of ethanol:chloroform 1:1, then twice with ammonium sulfate (first to 35%, then to 60% saturation). The final precipitate was resuspended in 20 mM sodium phosphate, pH 7.4, 0.5 M NaCl (buffer IMAC-A) and applied to Chelating Sepharose Fast Flow (Pharmacia, Uppsala) loaded with Zn^{2+}. After washing with two volumes of the starting buffer, adsorbed proteins were eluted by means of a linear gradient of IMAC-A and the same buffer, made 0.2 M in imidazol (= IMAC-B). Active fractions were pooled, concentrated by ammonium sulfate precipitation (80% saturation) and resuspended in the minimal volume of IMAC-A. This sample was applied to an appropriately sized column of Sepharose 6B-CL (Pharmacia, Uppsala) in the same buffer. The fractions containing the enzyme were pooled and slowly applied to a column of Red Sepharose (Pharmacia, Uppsala) in buffer IMAC-A. After thorough washing (three column volumes) with the starting buffer, elution was achieved with a linear gradient of IMAC-A: IMAC-A, 10mM in $NADP^+$. The determination of the NADPH-linked fluorescence intensity (excit. 340, emiss. 465 nm) showed that only 10% of the bound nucleotides remained in the reduced form.

Crystallization and X-ray data collection: The modified catalase A, the catA modC protein, was crystallized using the hanging drop vapor diffusion method and ammonium sulfate as precipitant at room temperature. The initial protein solution concentration of 28 mg/mL was mixed with an equal volume of a reservoir solution containing 2.5 M $(NH_4)_2SO_4$ and buffered with 0.2 M Tris at pH 8.5. Brown crystals appear in about one week and grow to their final size of about 0.5 × 0.5 × 0.8 mm, in approximately one month. These crystals show a bipyrimidal morphology with a hexagonal base, diffract isotropically till about 2.0 Å resolution in a conventional X-ray source and were stable to X-ray radiation for several hours. The space group, characterized from oriented oscillation images with the DENZO package (Otwinowski, 1993), was hexagonal $\mathbf{P6_122}$ with unit cell parameters $\mathbf{a} = \mathbf{b} = 184.3$ Å and $\mathbf{c} = 305.5$ Å (the enantiomorph space group, $\mathbf{P6_522}$, was discarded during the molecular replacement analysis).

Diffraction data were collected with a big MarScanner detector (30 cm) and using CuKα radiation from a rotating anode generator Siemens operating at 40 kV and 100 mA. The X-ray beam of 300 × 300 microns was focussed and collimated with a double mirror system (Frankc's mirrors). A spectrum was collected from a single crystal at room temperature with the longest **c** crystal axis close to the spindle axis using oscillation ranges of 1.0° with a crystal to detector distance of 280 mm. Data were processed and scaled with the DENZO package, giving a completeness of 76% (53386 independent reflexions) in the resolution shell 3.2 to 20.0 Å and an internal linear **R** factor of 7.9% with a mean $I/\sigma(I)$ of 8.5. Main difficulties to obtain high-resolution data were due to the large unit cell size, in particular, the length of the **c** axis.

Molecular replacement solution and crystal packing: Density packing considerations were consistent with the presence from four to six catalase subunits in the asymmetric unit of the crystal with specific volumes (V_M) of 3.1 and 2.1 Å^3/dalton, respectively (Matthews, 1968). No clear solutions for the rotation or the translation functions could be found when using as searching models the structure of the BLC monomer and the data set described above. Instead, a clean signal, corresponding to the strongest peak in the rotation, was obtained when using a modified BLC tetramer model with the molecular replacement program AMORE (Navaza, 1992). In the modified BLC structure about 150 residues per subunit, corresponding to regions without sequence homology, had been omitted. The longest fragments deleted from the BLC model belonged to the amino terminal arm and to the wrapping domain and included the coordinates from Asn 3 to Leu 26 and from Ala 383 to Leu 458, respectively. The coordinates of side chain atoms from residues Cis 459 until Asn 500 were also omitted. Rigid body refinement allowing the independent movement of each subunit decreased the R factor to 42%, for all data in the resolution shell 4.0 to 15.0 Å, without introducing any significant deviation from the 222 molecular symmetry. Crystal packing analysis did not show empty cavities large enough to contain other catalase molecules, confirming that only four subunits, a single catA modC molecule, are located in the asymmetric unit of the crystal with an estimated solvent volume content of 61% (Matthews, 1968). Molecules pack along the crystal sixfold axis forming a solvent channel with a diameter larger than 25 Å (Fig. 1). No major steric problems have been detected for the BLC-like model. However, the extensive intermolecular contact areas observed between neighbor molecules would prevent the presence of an extra domain in a disposition similar to the carboxy terminal domain found in PVC (Melik-Adamyan et al., 1986). The intermolecular interactions of two of the four subunits in the asymmetric unit involve the molecular regions in the vicinity of the NADPH binding site located in BLC (Fita et al., 1986) and in the bacterial catalase from *Proteus mirabilis* (Gouet et al., 1995). This observation suggests that heterogeneity in the occupancy of the nucleotide can be a major hindrance for crystallization.

Crystals characterized in this work allow high-resolution studies of the catalase A structure. However, mainly due to the large unit cell size, accurate data collection at high resolution requires an X-ray synchrotron source providing an intense, non-divergent beam. The preliminary analysis of the structure found confirms that catalase A presents important differences with PVC the only other fungal catalase structure available. Instead, catalase A retains some of the basic features of the mammalian catalases including the presence of an NADPH binding site in the surface of every subunit and the absence of a C-terminal domain.

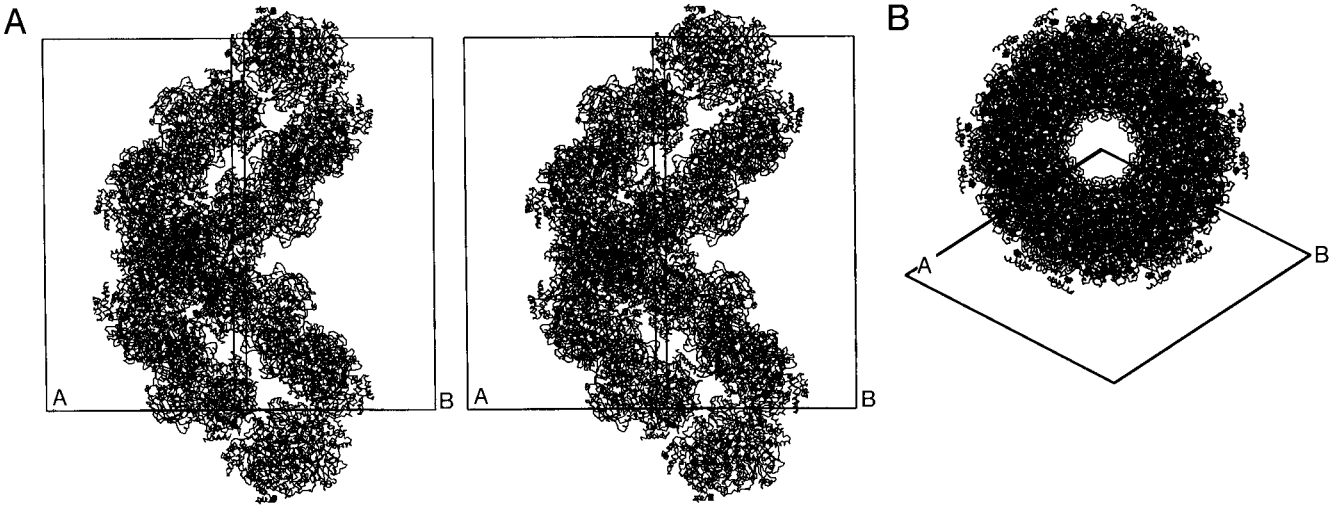

Fig. 1. A: Stereo view of crystal packing perpendicular to the crystallographic **c** axis. A single molecule, a tetramer with accurate 222 symmetry, is contained in the asymmetric unit of the crystal. The 12 molecules generated by the crystal symmetries without translations are represented. The extensive contact areas between neighbor molecules would prevent the presence of an extra domain and, for two of the four subunits, involve residues in the vicinity of the NADPH binding site (see the text). **B:** A large solvent channel around the sixfold axis is apparent when the packing is viewed down the **c** crystal axis.

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